DISSERTATION ON

STUDY OF LIPID PROFILE IN

PREGNANCY INDUCED HYPERTENSION

SUBMITTED FOR

M.D. BRANCH - XIII

(BIOCHEMISTRY)

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

FOR THE EXAMINATION IN MARCH 2008

CERTIFICATE

This is to certify that **Dr. P. JOSEPHINE LATHA,** is a bonafide student of **MD., Biochemistry** has done her dissertation in the Department of Biochemistry, Thanjavur Medical College, Thanjavur.

Date :

Head of the Department

Place : Thanjavur

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CONTENTS

LIPID PROFILE IN PREGNANCY INDUCED HYPERTENSION

INTRODUCTION

Comprehensive antenatal care should ensure a healthy pregnancy with an outcome of a healthy mother and a healthy fetus with a good birth weight. Birth weight is not only the indicator of the health of the new born child but also it predicts the future health of the child. Low birth weight child is prone to suffer from diabetes, hypertension, and coronary vascular disorders in their later life. [1]

Pre eclampsia, a disease of pregnant women is one of the leading causes of maternal and fetal morbidity and mortality[2] and it occurs in 2-7% of all pregnancies. [3]

The incidence of pregnancy induced hypertension in India ranges from 5-15%

In Primi's - 16%

In Multi's- 7%

It causes IUGR leading to low birth weights.[1,5] It increases maternal mortality by 10-15% and perinatal mortality and morbidity by 15 to 25%[1].

With high blood pressure, there is an increase in the vascular resistance which may hinder blood flow in different organ systems in the expectant mother including liver, kidneys, brain, uterus and placenta [3, 4]

Complications of PIH are

1. HELLP syndrome[7]- which includes Hemolysis, elevated liver enzymes, low platelets leading to acute fatty liver and liver rupture.
2. Renal failure / Impairment
3. Pulmonary edema
4. Disseminated intravascular coagulation
5. Eclampsia
6. Placental abruption

Early identification of women at risk for PIH may help in preventing the complications of the disease.

According to the norms of American college of obstetrics and Gynaecologist, PIH[6] is defined as

1. Systolic Blood Pressure $\geq$ 140mmHg
2. Diastolic Blood Pressure $\geq$ 90mmHg
3. Increase of $\geq$ 30mgHg in systolic pressure
4. Increase of $\geq$ 15mmHg in diastolic Blood Pressure [5]

in a previously normotensive woman.

If proteinuria develops along with increased Blood pressure, then it comes under pre eclampsia.

Pathophysiology of PIH:

The most important determinants of blood pressure are[1]

1. Cardiac output
2. Peripheral vascular resistance

Even when cardiac output is increased[7] during normal pregnancy there is a relative decrease in blood pressure due to the decrease in peripheral vascular resistance. But in PIH there is increased resistance which is brought about by

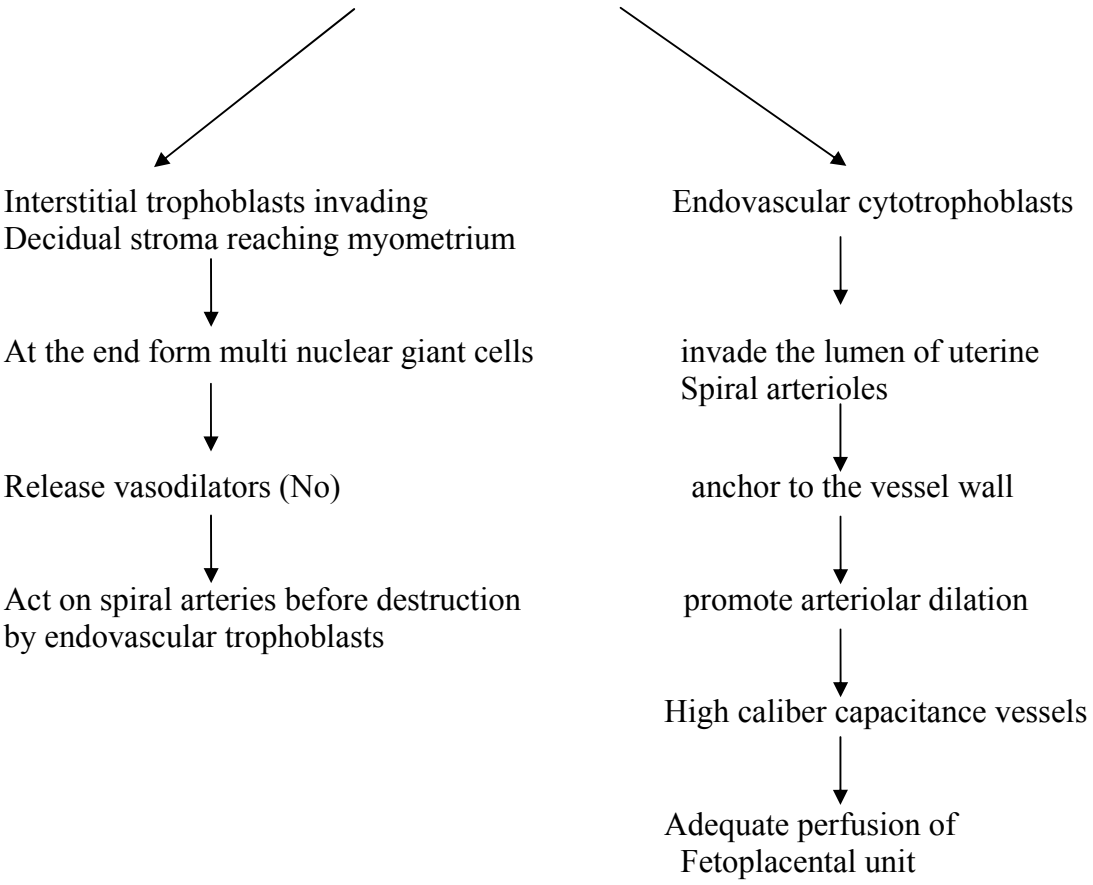
1. Increased response to vasopressors[8].
2. Altered lipid synthesis leading to decrease in the ratio of PGI2/TXA2 and antioxidants/lipid peroxidases[8,17]. Balance between peroxide generating and peroxide removing mechanisms may control the rate of generation of prostaglandins. Prostacyclin, a potent vasodilator and antiaggregatory agent is synthesised in vascular endothelium. This is balanced by thromboxane, a potent locally acting vasoconstrictor and proaggregatory agent. Lipid peroxides inhibit prostacyclin synthase but do not influence thromboxane synthase[55,65]. leading to decrease in vasodilator effect. Inhibition of prostacyclin synthesis leads to the formation of foam cells and so atherogenesis. Acute atherosis, including the presence of foam cells is a feature of placental bed biopsy specimens from women with pre-eclampria[74].
3. Changes in the local factors like NO, endothelins.

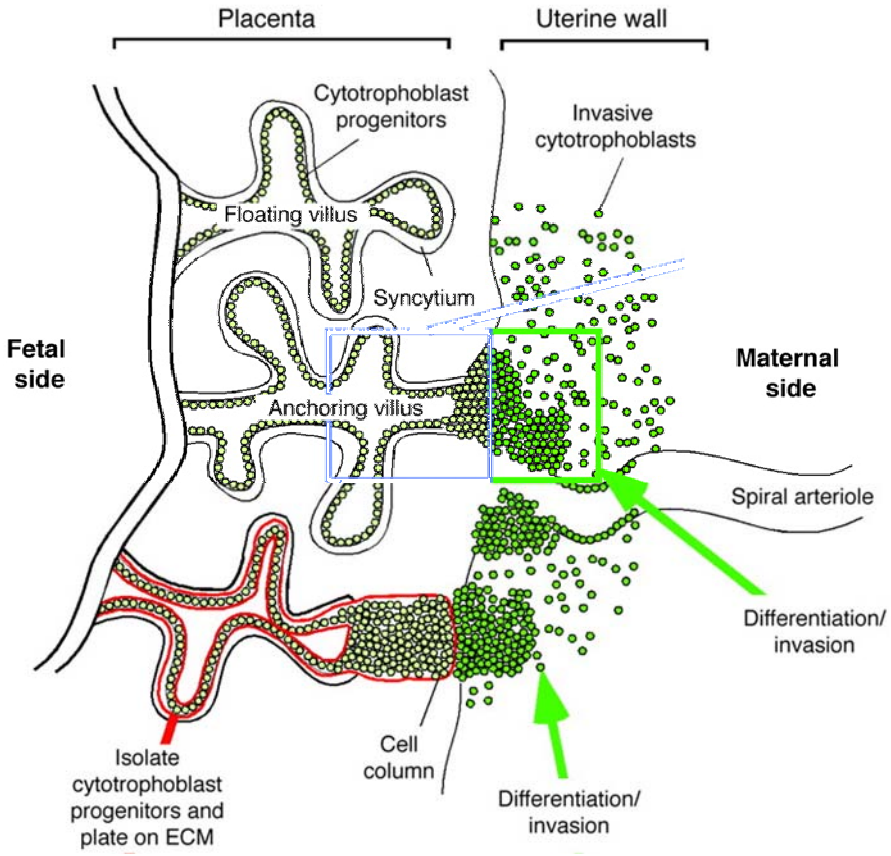
There is an accumulated evidence stating that abnormal placentation is one of the initial events leading to this disease. The main feature is inadequate trophoblastic invasion of maternal spiral arterioles[2,3,7,9] and the arteries retain their musculoelastic walls and thus respond to the vasoconstrictors produced (Eg.) leukotrienes, lipid peroxidases.

Factors involved in abnormal placentation are :

Intrinsic factors acting in concert with external extrinsic maternal uterine factors.

Intrinsic factors ⟶ abnormal biology of extra villous trophoblasts.




Placenta
Uterine wall
Cytotrophoblast progenitors
Invasive cytotrophoblasts
Floating villus
Syncytium
Fetal side
Maternal side
Anchoring villus
Spiral arteriole
Differentiation/ invasion
Isolate cytotrophoblast progenitors and plate on ECM
Cell column
Differentiation/ invasion

In pre-eclampsia :

Impaired interstitial trophoblasts invasion and failure of vascular Invasion ⟶ narrow bore high resistance spiral arterioles ⟶ inadequate Perfusion of fetoplacental unit.

Extrinsic uterine maternal factors are :

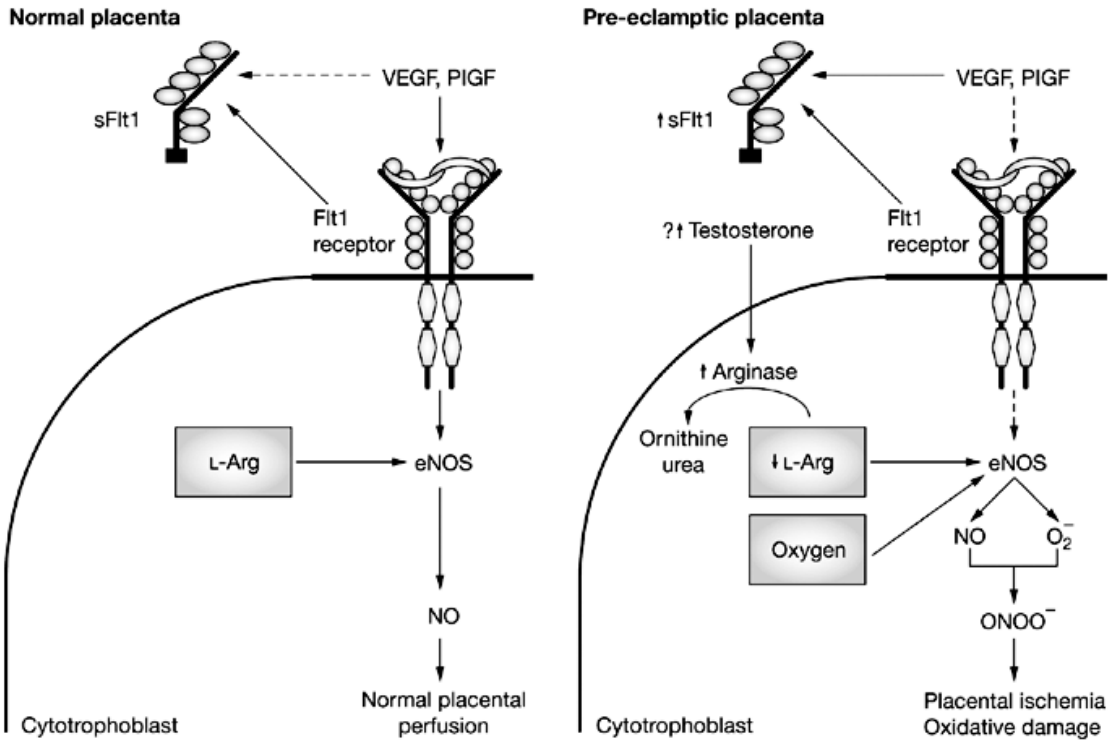
1. Impaired decidual remodelling[2,9]
2. Impaired function of uterine natural killer cells. Interaction of inhibitory killer cell immunoglobulin receptors or uterine natural killer cells with trophoblastic human leukocyte Antigen C leads to excessive inhibition of uterine natural killer cell activity and so decreased invasion of trophoblasts leading to defective trophoblast remodeling of maternal blood vessels.
3. Maternal endothelial failure to express adhesion molecules. (i.e.) impairment in transformation of epithelial tissue to endothelium as in normal pregnancy.

Biochemical aspects of placental Ischaemia[2]

1. Binding of vascular endothelial growth factor and placental growth factor to the Fms-like tyrosine kinase 1 receptor on cytotrophoblasts stimulates production of nitric oxide by endothelial nitric oxide synthase. Interaction of vascular endothelial growth factor and placental growth factor with the Fms-like tyrosine kinase 1 receptor is inhibited by soluble Fms-like tyrosine kinase 1. Normal placenta with sufficient tissue L-arginine

sustains adequate generation of nitric oxide by endothelial nitric oxide synthase (left panel).

2. By contrast, in pre-eclampsia, increased levels of soluble Fms-like tyrosine kinase1 inhibit activation of endothelial nitric oxide synthase, by the Fms-like tyrosine kinase 1 receptor. At the same time, excessive arginase II expression reduces the placental L-arginine concentration, causing endothelial nitric oxide synthase to preferentially facilitate superoxide anion production. Superoxide anion reacts with nitric oxide to form peroxynitrite, thus reducing the half-life of nitric oxide (right panel). This promotes abnormal placental perfusion and microvascular oxidative damage.



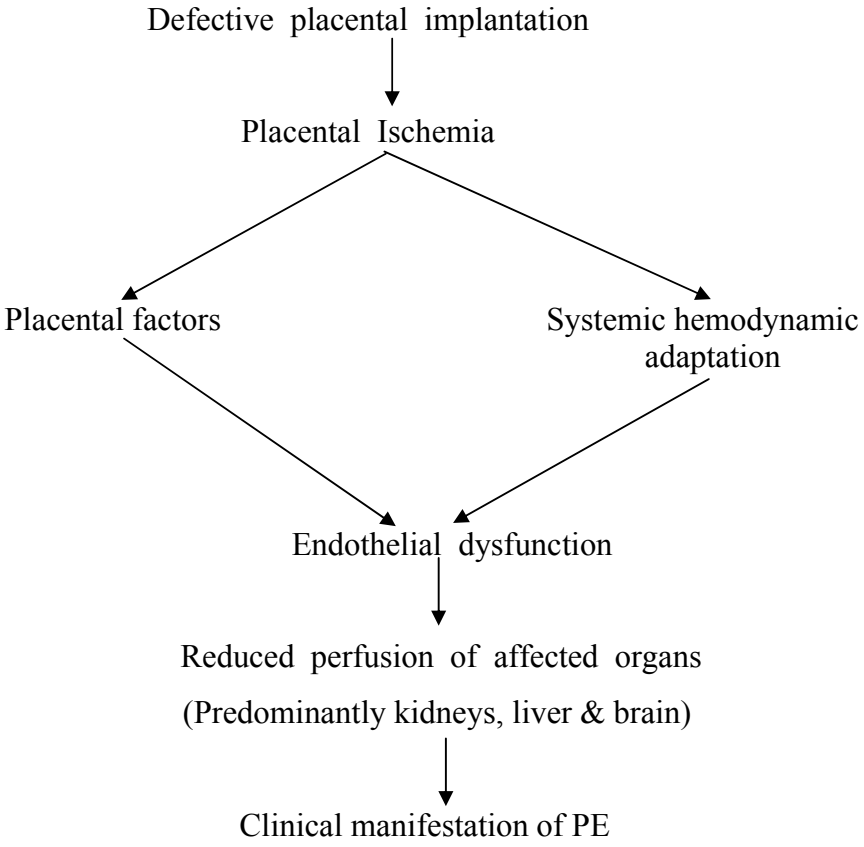
eNOS, endothelial nitricoxide synthase; Flt1, Fms-like tyrosine kinase 1; L-Arg, L-arginine; NO, nitric oxide; O2–, superoxideanion; ONOO–, peroxynitrite; PlGF, placental growth factor; sFlt1, soluble Fms-like tyrosine kinase 1;

MATERNAL LEVEL

The placental abnormalities described, have several maternal consequences.

2 Stages of Pre – Eclampsia[2]

Stage 1



Stage 2

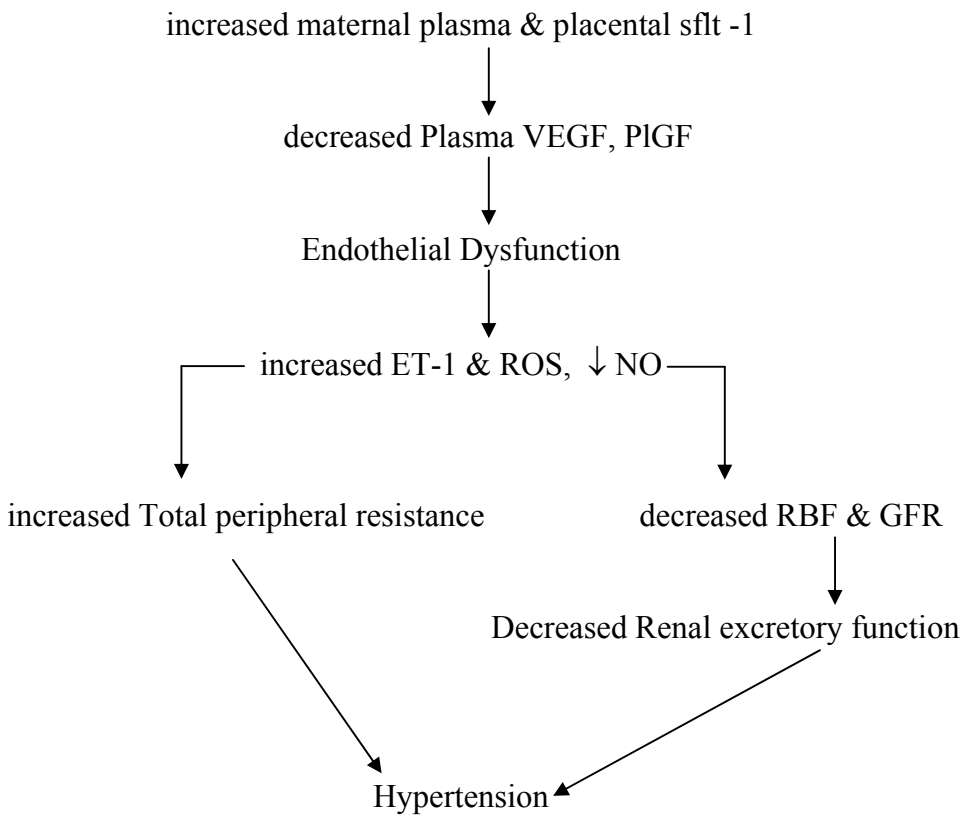
Endothelial dysfunction

Reduced perfusion of affected organs

(Predominantly kidneys, liver & brain)

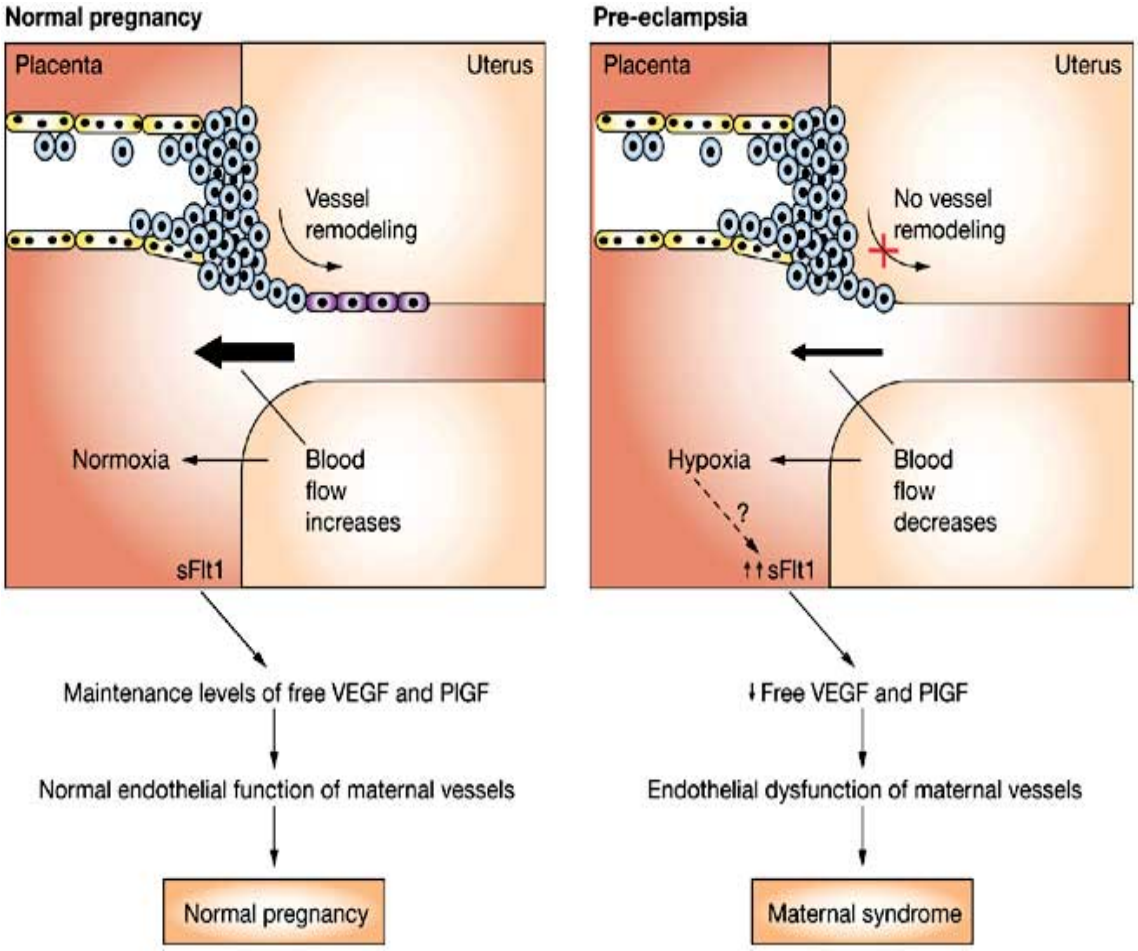
Clinical manifestation of PE

Reduced uterine perfusion pressure



Role of the soluble form of Fms-like tyrosine kinase 1 in the maternal syndrome of pre-eclampsia

Most of the Fms-like tyrosine kinase 1 produced by human placenta is a soluble form generated by alternative splicing . Soluble Fms-like tyrosine kinase 1 is released in large amounts into the blood. Soluble Fms-like tyrosine kinase 1 binds both vascular endothelial growth factor and placental growth factor, reducing their free levels in the blood by working as a soluble antagonist of both factors, and maintaining normal endothelial function of maternal vasculature (left panel). Pre-eclamptic placenta releases higher amounts of soluble Fms-like tyrosine kinase 1 than normal placenta (right panel),depriving the vasculature of kidney, liver, brain and other organs of essential maintenance signals, thereby triggering the maternal vascular dysfunction of pre-eclampsia.



PlGF, placental growth factor;sFlt1, soluble Fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor.

Unifying hypothesis of pre-eclampsia pathophysiology.

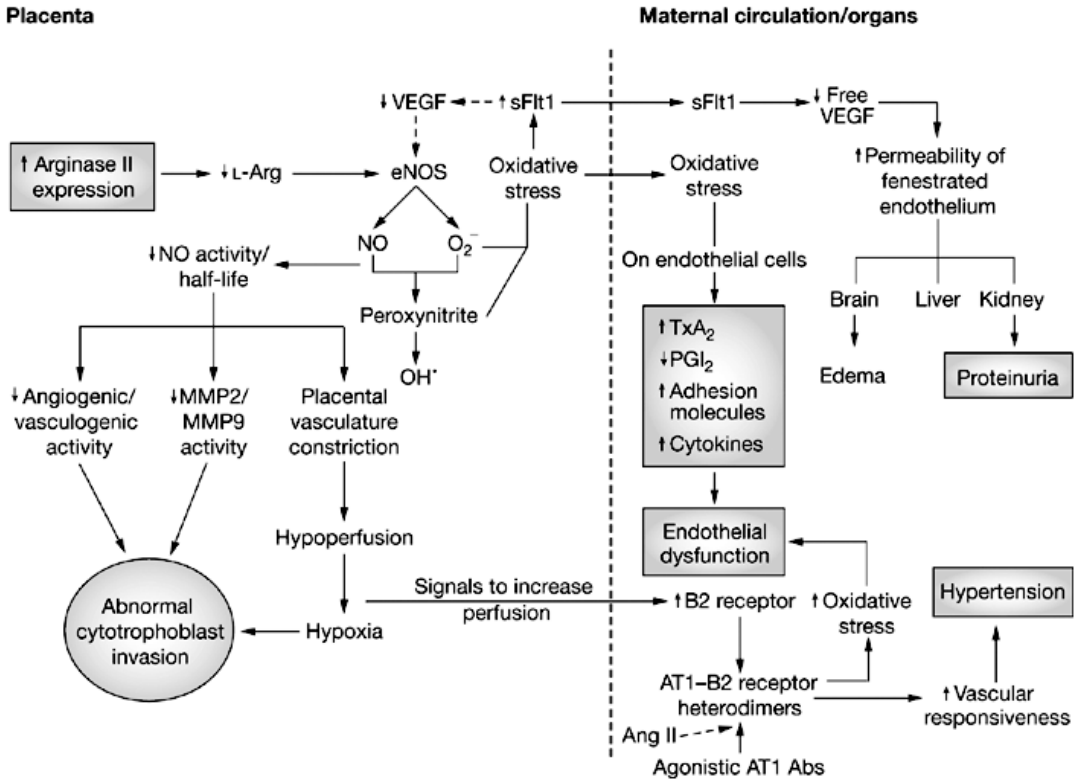
According to this, pre-eclampsia is a disease of placentation triggered by reduced activity or half-life of nitric oxide secondary to low placental L-arginine concentration, which is in turn related to excessive arginase II expression. Low L-arginine levels stimulate endothelial nitric oxide synthase to generate reactive oxygen species (e.g. peroxynitrite and hydroxyl radical) and locally exacerbate oxidative stress. Reduced flow through the placental L-arginine–nitric oxide pathway is also consistent with the higher resistance and hypoperfusion of the fetal–placental circulation. In response to the restricted placental blood flow and consequent hypoxia, the placenta releases into the maternal circulation a number of factors including soluble Fms-like tyrosine kinase 1 and reactive oxygen species, which initiate the vascular dysfunction characteristic of the maternal syndrome.

Soluble Fms-like tyrosine kinase 1 is present at high concentrations in the blood of pre-eclamptic women,and administration of soluble Fms-like tyrosine kinase 1 to animals produces a maternal syndrome resembling that of pre-eclampsia.

In a further attempt to ameliorate perfusion of the placenta as well as of the maternal organs involved in pre-eclampsia, upregulation of the B2 receptor for the vasodilator bradykinin occurs. The B2 receptors heterodimerize with the angiotensin II type 1 receptor, thereby increasing vascular and inflammatory

responsiveness to angiotensin II, paradoxically reducing systemic organ perfusion and promoting generation of reactive oxygen species.

Concomitant production of agonist angiotensin II type 1 receptor autoantibodies also contributes to oxidative stress. A positive-feedback loop is initiated that eventually results in the full-blown clinical syndrome of pre-eclampsia.



Ang II, angiotensin II; AT1, angiotensin II type 1 receptor; eNOS, endothelial nitric oxide synthase;

L-Arg, L-arginine; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; NO, nitric oxide;

O2–, superoxide anion; OH•, hydroxyl radical; PGI2, prostacyclin; sFlt1, soluble Fms-like tyrosine kinase 1;

TxA2, thromboxane A2; VEGF, vascular endothelial growth factor

Lipid metabolism in pregnancy

Normal pregnancy is hyperlipidemic[10]

3 fold increase in TGL and fatty acids.

50% increase in LDL

HDL is also increased.

One of the reasons for this increase may be due to the reduction in intestinal motility which allows more time for the absorption of fat. Reduction of enterohepatic circulation with increased excretion of cholesterol in the bile also leads to alteration in lipid profile[10].

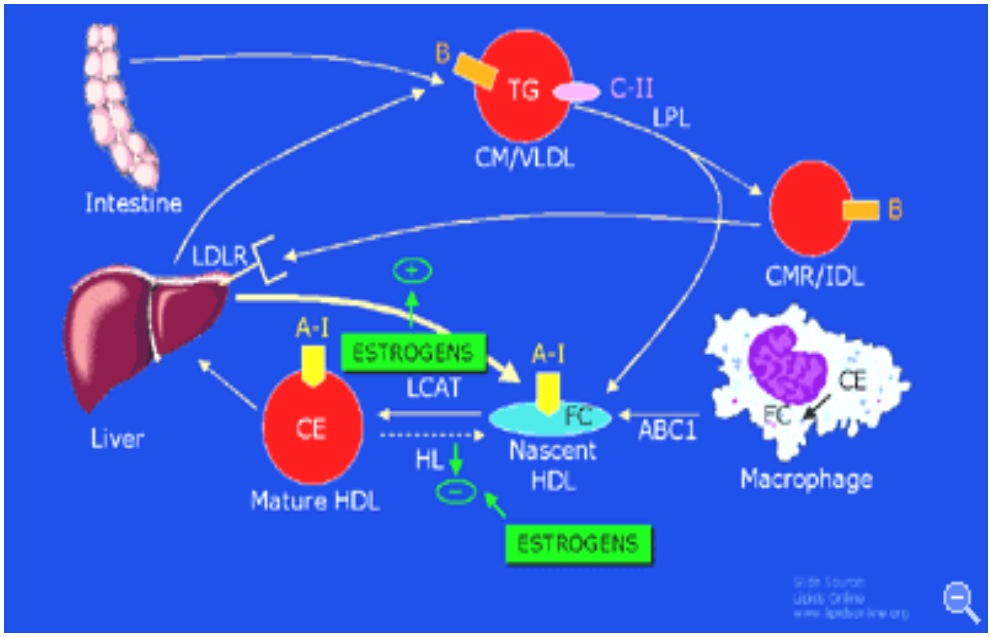
There is reduction in hepatic lipase & LPL during pregnancy which may lead to decrease in the clearance of VLDL and hence increase in VLDL (Kinnernen 1980, Herrera et al 1987)[10], though the Hypertriglyceridemia is principally due to increased entry of TGL –rich Lipoprotein into the circulation (Knopp et al 1982) Normal pregnancy is a state of hyperestrogenemia.

Estrogen results in increase in HDL level and TGL level, decrease in LDL level.

1. Increased apolipoprotein A-I production
   Inhibition of activity of hepatic lipase
   } Increased production of mature HDL

2. Increased rate of VLDL production and Decrease in clearance of VLDL } Hypertriglyceridemia

3. Increased LDL receptor and increased LDL removal in the liver } Decrease in LDL level.



4. Decrease in Lp (a)

5. Decrease in apo B

6. Increased endothelial production of NO and prostacyclin ⟶ vasodilatory effect.

7. Inhibits oxidation of LDL and protects against toxic effects of oxidized LDL on endothelium.

Lipid metabolism in PIH

Pre-eclampsia is associated with insulin resistance & a dramatic increase in FFA (antedating clinical expression of the disorder) and plasma TGL well above that seen in normal pregnancy. (Lorenzen et al 1995)[10,59,60] The changes in lipoprotein subfraction are compatible with changes seen in coronary artery disease (Kaaja et al 1995, sattar et al 1997)

1. The dramatic increase in TGL → increase in VLDL
   small changes in LDL
   decrease in HDL

   → Vascular damage and dysfunction directly &

   → increased oxidative stress potentiating insulin resistance – Vascular dysfunction indirectly(Hayman et al 1999& saltar & Green 1999)[18]

Hypoestrogenemia also leads to the above changes[8] A significantly higher level of LDL was also reported by many workers in third trimester of gestational proteinuric hypertension[8,19,20,21].

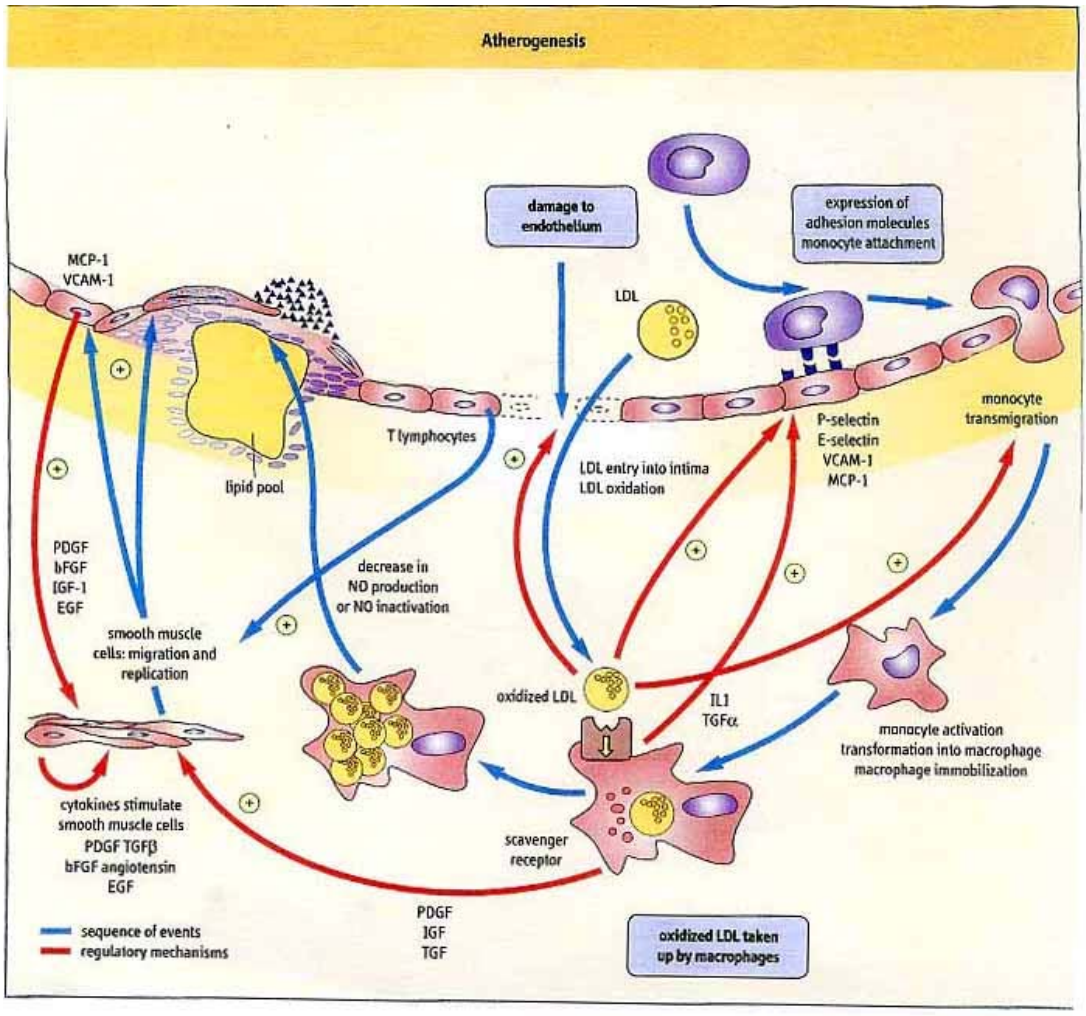
Hypoestogenemia, predominance of smaller and denser serum LDL and significant concentration of soluble vascular cell adhesion molecule-1 are important contributors for endothetial dysfunction. in PIH [(8,19,22,23)]

Development of atherosclerosis by hypertriglyceridemia in Pre eclampsia

In preeclampsia there is a higher risk of placental vasculopathy [24,25] There is evidence of chronic inflammation, hypercoagulability[24-26] and endothelial dysfunction[27] in persons with metabolic syndrome of which hypertriglyceridemia is a major feature. In lipid –mediated endothelial dysfunction an essential step is oxidation of low-density lipoprotein[24,28,29]. The markers of LDL oxidation and circulating serum free fatty acids[24,30] are increased in women with pre- eclampsia.

The atherogenesis itself may be initiated by hypertriglyceridemia. Monocytes first adhere to endothelial cells and then transmigrate into the vascular intima[32,61] For this adhesion which is the initial event, leukocyte and vascular cell adhesion molecules such as selectins, integrins, VCAM -1 and ICAM-1 play a critical role. These adhesion molecules are significantly increased in the serum of patients with severe hypertriglyceridemia and studies have shown that these CAMS are expressed at increased levels in atherosclerotic plaques[62-65].

Since Triglyceride related vasculopathy may be one of the etiologic factors and positively correlated with the development of pre eclampsia and also may influence a future pregnancy as well as a woman's long term risk of cardiovascular disease. It is worthwhile to explore the metabolism and transport of various subfractions of lipoproteins in pregnant women from 20 weeks of gestation who have developed increase in blood pressure.


Atherogenesis
damage to endothelium
expression of adhesion molecules monocyte attachment
MCP-1
VCAM-1
LDL
T lymphocytes
lipid pool
LDL entry into intima
LDL oxidation
P-selectin
E-selectin
VCAM-1
MCP-1
monocyte transmigration
PDGF
bFGF
IGF-1
EGF
decrease in NO production or NO inactivation
smooth muscle cells: migration and replication
oxidized LDL
IL1
TGFα
monocyte activation transformation into macrophage macrophage immobilization
cytokines stimulate smooth muscle cells
PDGF TGFβ
bFGF angiotensin
EGF
scavenger receptor
PDGF
IGF
TGF
sequence of events
regulatory mechanisms
oxidized LDL taken up by macrophages

OBJECTIVE OF THE STUDY

To evaluate the implication of pregnancy induced hypertension on maternal plasma lipid profile by comparing with normotensive pregnant women.

MATERIALS AND METHODS

The study was carried out in a tertiary care centre, Raja Mirasudar Hospital, Thanjavur, attached to our Medical college.

100 women with pregnancy induced Hypertension and 100 normotensive pregnant women as controls are included in the study.

The diagnosis of PIH was done as per the norms of American college of Obstetrics and Gynecologists.

The definition includes

1) Systolic Blood pressure ≥140 mm/Hg
2) Diastolic Blood pressure ≥90 mm/Hg

Or

3) increase of ≥30 mm/Hg in Systolic pressure

Or

4) increase of ≥15 mm/Hg in Diastolic pressure

All the participants were inquired by a questionnaire

1) Name of the person
2) Age of the person
3) Address
4) Complaints
5) Last Menstrual period

6) Gravida

7) Para

8) Head ache

9) Inability to tolerate Bright light Blurred vision

10) Double vision

11) Nausea, Vomiting

12) Right sided upper Quadrant pain

13) Epigastric pain

14) Dyspnea

15) Oliguria

16) Bleeding tendency

17) Sudden weight gain

18) Edema

19) Tiredness

Previous pregnancy with PIH:

1) Diabetic

2) Hypertensive

3) Hyperlipidemia

4) Renal Disease

5) Hypothyroid

6) Hepatic Disease

FAMILY HISTORY

1) PIH
2) Twins
3) Hypertension
4) Diabetic

DRUG HISTORY:

1) Thiazides
2) Retinoid:
3) ART:
4) Estrogen:
5) β-blockers:
6) Progestin:
7) Glucocorticoids:

INCLUSION CRITERIA:

1) Any age 20-45
2) Any Gravida
3) After 20 Wks of pregnancy
4) BP≥140/90 for PIH
5) ≤120/80 control

EXCLUSION CRITERIA

1) Known Diabetic, Hypertensive
2) Hyperlipidemic before 20 wks.
3) Edema
4) Proteinuria, oliguria
5) Hepatic disease
6) Involvement of other Organs.

Clinical examination of participants were carried out to rule out Diabetes, Hypertension, Hyperlipidemia before 20 wks, edema, proteinuria, oliguria, Hepatic disease, involvement of other Organs.

Fasting blood specimen was collected from each participant.

METHODOLOGY

DETERMINATION OF GLUCOSE IN SERUM

GOD/POD METHOD

PRINCIPLE

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Contents

L1: Glucose reagent; 4x250ml

L2: Buffer reagent: 10 ml

S:lucose standard(100 mg/dl):5ml

Reagent preparation:

2.5ml of Buffer reagent (L2) was added to 250ml of distilled water

The contents of one bottle of glucose reagent (L1) was emptied into it, and mixed by gentle swirling and allowed to stand at room temperature for 30 minutes.This working reagent is stable for 60 days when srtored at 2-8° C.

SAMPLE MATERIAL - Serum

PROCEDURE

Wave length/Filter:505nm(Hg 546 nm)Green

Temperature: 37° C /RT

Light path:1cm

The working reagent, distilled water, standard and sample were pipetted into clean dry test tubes labelled as Blank (B), Standard (S),and Test(T) as follows :

Addition Sequence	**B (ml)**	**S (ml)**	**T (ml)**
Working Reagent	1.0	1.0	1.0
Distilled water	0.01	---	---
Glucose standard	--	0.01	---
Sample	--	--	0.01

Mixed well and Incubated at 37° C for 10 minutes. The absorbance of the standard (Abs.S) and Test sample (Abs.T) were measured against the blank, within 60 minutes at 505nm.

Calculations:

$$\text{Total glucose in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Linearity:

This procedure is linear up to 500mg/dl.

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	505nm
Incubation Temp	:	37°C/R.T
Incubation Time	:	10 min/30 min
Sample Vol	:	10µL
Reagent Vol	:	1.0mL
Std. Concentration	:	100mg/dL
Zero Setting With	:	Reagent Blank
Linearity	:	500 mg/dl

Reference value

Serum: Fasting: 74-106 mg/dl

ESTIMATION OF CHOLESTEROL

ENZYMATIC METHOD

PRINCIPLE

$$\text{Cholesterol Ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4 - H_2O$$

The concentration of Cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500 nm.

REAGENTS

Reagent 1 (Enzymes/ Chromogen)

Cholesterol Esterase	≥ 200U/L
Cholesterol Oxidase	≥ 250U/L
Peroxidase	≥ 1000U/L
4-Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer):

Pipes buffer, pH 6.90	50 mmol/L
Phenol	24mmol/L
Sodium Cholate	0.5mmol/L

Standard (Cholesterol 200 mg/dL):

Cholesterol	2 g/L

STORAGE & STABILITY OF THE REAGENTS

When stored at 2° C-8° C and protected from light, the reagents are stable until the expiry dates stated on the labels.

REAGENT RECONSTITUION

The reagents are allowed to attain room temperature. The contents of one bottle of **reagents 1** were dissolved with one bottle of **reagent 1A** and mixed by gentle swirling.

RECONSTITUTED REAGENT STORAGE & STABILITY

The reconstituted reagent is stable for 3 Months when stored at 2°C-8°C.

PROCEDURE

The samples and the reconstituted reagent were brought to room temperature prior to use.

The following general system parameters were used with this kit:

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	500 nm (492-550)
Flowcell Temp	:	30° C
Incubation	:	5 Min. at 37°C
Sample Vol	:	10µL
Reagent Vol	:	1.0mL
Std. Concentration	:	200 mg/dL
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and the sample were dispensed in to test tubes as follows

	Blank	**Standard**	**Test**
Reconstituted	1mL	1mL	1mL
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubated for 5 minutes at 37° C, mixed and read at 500nm

Linearity:

The method is linear up to 500 mg/dL.

Reference value for Cholesterol

Serum/Plasma: Female 20 – 24 yrs : 122-216mg/dl
25 – 29yrs : 128-222 mg/dl
30-34yrs : 130 – 230 mg/dl

ESTIMATION OF TRIGLYCERIDES

ENZYMATIC COLORIMETRIC METHOD

PRINCIPLE

$$\text{Triglycerides} + H_2O \xrightarrow[\text{Lipase}]{\text{Lipoprotein}} \text{Glycerol} + \text{Fatty Acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Kinase}]{\text{Glycerol}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dehydroxyacetone Phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{ADPS} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_2O$$

GPO = Glycerol -3- Phosphate Oxidase

ADPS = N-Ethyl-N- Sulfopropyl -n-anisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the Triglycerides concentration in the sample and is measured at 546 nm

REAGENTS

Reagent 1 (Enzymes/ Chromogen)

Lipoprotein Lipase	≥ 1100U/L
Glycerol Kinase	≥ 800U/L
Glycerol-3- Phosphate Oxidase	≥ 5000U/L
Peroxidase	≥ 350U/L
4-Aminoantipyrine	0.7 mmol/L
ATP	0.3 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 7.50	50 mmol/L
ADPS	1 mmol/L
Magnesium salt	15 mmol/L

Standard (Triglycerides 200 mg/dL)

Glycerol (Trig. equivalent)	2 g/L

REAGENT RECONSTITUION

The reagents are allowed to attain room temperature. The contents of one bottle of **reagents 1** were dissolved with one bottle of **reagent 1A,** and mixed by gentles swirling and used after 5 minutes.

RECONSTITUTED REAGENT STORAGE & STABILITY

The reconstituted reagent is stable for 6 weeks when stored at 2°C-8°C.

PROCEDURE

The samples and the reconstituted reagent were brought to room temperature prior to use.

The following general system parameters were used with this kit:

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	546 nm (520-570)
Flowcell Temp	:	30° C
Incubation	:	5 Min. at 37°C
Sample Vol	:	10µL
Reagent Vol	:	1.0mL
Std. Concentration	:	200 mg/dL
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and sample were dispensed in to test tubes as follows :

	Blank	Standard	Test
Reconstituted reagent	1mL	1mL	1mL
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubated for 5 minutes at 37° C. Mixed and read at 546nm. The final colour was stable for at least 30 minutes.

Linearity:

The method is linear up to 1000 mg/dL.

Reference value for Triglycerides:

Serum/ Plasma

Females: 20 – 29yrs = 37 - 144 mg/dl

30 – 39yrs = 39 - 176 mg/dl

ESTIMATION OF HDL-CHOLESTEROL

PHOSPHOTUNGSTATE METHOD

PRINCIPLE

Chylomicrons, VLDL (Very Low Density Lipoproteins) and LDL fractions in serum or plasma are separated from HDL by precipitating with Phosphotungstic Acid and Magnesium Chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, is assayed with enzymatic cholesterol method, using Cholesterol Esterase, Cholesterol Oxidase, Peroxidase and the chromogen 4- Aminoantipyrine/Phenol.

REAGENTS

Reagent 1(Enzymes/Chromogen)

Cholesterol esterase	≥ 200 U/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥ 1000 U/L
4-Aminoantipyrine	0.5mmol/L

Reagent 1A (Buffer):

Pipes buffer, pH 6.9	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5mmol/L

Reagent 2 (Precipating Reagent)

Phosphotungstic Acid	2.4 mmol/L
Magnesium Chloride	39 mmol/L

Standard (HDL Cholesterol 50mg/dL):

Cholesterol	0.5g/L

REAGENT RECONSTITUTION:

The reagents are allowed to attain the room temperature. The contents of one bottle of **reagent 1 is dissolved into** one bottle of **reagent 1A,** and mixed by gentle swirling till completely dissolved and used after 5 minutes

RECONSTITUITED REAGENT STORAGE & STABILITY

The reconstituted reagent was stable for 3 months when stored at 2°C-8 °C.

PROCEDURE

The samples, the participating reagent 2 and the reconstituted reagent were brought to room temperature prior to use.

I. PRECIPITATION

The sample and precipitating reagent were dispensed into Centrifuge Tube as follows:

	Test
Sample	0.20 mL(200µL)
Precipitating Reagent 2	0.20 mL(200µL)

Mixed well and centrifuged at 1500 g or 3500-4000 rpm for 10 min. The clear supernatant was separated immediately and determined the Cholesterol content as for total cholesterol estimation.

II CHOLESTEROL ASSAY

The following general system parameters were used with this kit:

General System Parameters

Reaction Type	:	End point
Reaction Slope	:	Increasing
Wavelength	:	500 nm (492-550nm)
Flow cell Temp	:	30°C
Incubation	:	5Min 37°C
Sample Vol (Supernatant)	:	20µL
Reagent Vol	:	1.0 mL
Std.Concetration	:	100mg/dL(The Std.of 50 mg/dL is to be fed as 100 mg/dL to account for the dilution of sample in the precipitation step)
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and supernatent were dispensed into test tubes as follows:

	Blank	**Standard**	**Test**
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	20µL	--
Supernatant	-	-	20µL

Incubate for 5 minutes at 37°C, mixed and read at 500nm.

Reference value in HDL-Cholesterol:

Serum/Plasma; Female : 20-24 yrs : 33 – 79 mg/dl

25 – 29yrs : 37-83 mg/dl

30-34 yrs : 36-77 mg/dl

ESTIMATION OF LDL CHOLESTEROL BY

FRIEDWALD EQUATION

[LDL CHOLESTEROL]= [Total cholesterol] -[HDL cholesterol] -

[Triglyceride/5], all concentrations are in mg/dl.

VLDL = Triglyceride/5

Reference Value :

Female = 20 - 24 yrs : 57 - 159 mg/dl

25 - 29 yrs : 71 - 164 mg/dl

30 - 34 yrs : 70 - 156 mg/dl

ESTIMATION OF URIC ACID

ENZYMATIC METHOD

PRINCIPLE

Uric acid is converted by uricase into allantoin and hydrogen peroxide which in presence of peroxidase (POD) oxidises the chromogen to a red coloured compound which is read at 500 nm (492-550).The final colour of the reaction is stable for 15 minutes.

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{POD}} \text{Red quinone} + H_2O + HCl$$

DHBS=3.5-Dichloro-2-Hydroxybenzene Sulfonic Acid

REAGENTS

Reagent 1(Enzymes/Chromogen)

Uricase	≥ 60 U/L
Peroxidase	≥ 660 U/L
4-Aminoantipyrine	0.23mmol/L

Reagent 1A (Buffer)

Phosphate buffer, pH 7.5	50 mmol/L
DHBS	2 mmol/L

Standard (Uric Acid 6mg/dL)

Uric Acid : 0.06g/L

STORAGE & STABILITY OF THE REAGENTS

When stored at 2°C-8 °C and protected from light, the reagents are stable until the expiry dates stated on the labels.

REAGENT RECONSTITUTION:

The reagents are allowed to attain the room temperature. The contents of one bottle of **reagent 1** is dissolved with one bottle of **reagent 1A**.

RECONSTITUITED REAGENT STORAGE & STABILITY

The reconstituted reagent was stable for 4 weeks when stored at 2°C-8 °C.

PROCEDURE

The sample and the reconstituted reagent were brought to room temperature prior to use. The following general system parameters were used with this kit:

General System Parameters

Reaction Type	:	End point
Reaction Slope	:	Increasing
Wavelength	:	510 nm (492-550)
Flow cell Temp	:	30°C

Incubation : 5Min.at 37°C

Sample Vol : 25µL

Reagent Vol : 1mL

Std.Concetration : 6mg/dL

Zero Setting With : Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and sample were dispensed into test tubes as follows :

	Blank	**Standard**	**Test**
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	25µL	--
Sample	-	-	25µL

Incubated for 5 min. at 37°C.Mix and read at 510nm

The final colour is stable for at least 15 minutes.

Linearity:

The method is linear up to 25 mg/dL.

Reference value in Serum/Plasma

Males : 3.5 - 7.2 mg/dL

Females : 2.6 - 6.0 mg/dL

RESULTS

LIPID PROFILE IN PIH														
Sl.No	**AGE**	**LMP (WEEKS)**	**BP**	**FBS**	**TC**	**TGL**	**HDL**	**LDL**	**VLDL**	**TC/HDL**	**TGL/HDL**	**HDL/VLDL**	**UA**	**LDL/HDL**
1	33	32	144/92	83.5	181	280	40	85	56	4.53	7.00	0.71	4.1	2.13
2	28	28	140/90	73.6	216	299	40	116.2	59.8	5.40	7.48	0.67	2.8	2.91
3	20	32	140/90	57	258	373	28	155.4	74.6	9.21	13.32	0.38	4.4	5.55
4	25	28	140/100	110	214	268	38	122.4	53.6	5.63	7.05	0.71	3.1	3.22
5	27	32	140/90	79	249	328	36	147.4	65.6	6.92	9.11	0.55	4.7	4.09
6	23	32	140/90	82	252	290	40	154	58	6.30	7.25	0.69	4.1	3.85
7	28	32	140/94	104	210	520	35	71	104	6.00	14.86	0.34	3.8	2.03
8	28	22	140/90	83	261	290	42	161	58	6.21	6.90	0.72	3.7	3.83
9	22	20	140/90	98	190	400	37	73	80	5.14	10.81	0.46	3	1.97
10	25	32	150/120	91	232	580	40	76	116	5.80	14.50	0.34	7.6	1.90
11	26	32	140/90	102	188	540	34	46	108	5.53	15.88	0.31	8.5	1.35
12	27	32	140/90	86	193	440	30	75	88	6.43	14.67	0.34	4.4	2.50
13	24	28	140/100	100	244	480	35	113	96	6.97	13.71	0.36	4.7	3.23
14	27	24	140/90	81	181	297	30	91.6	59.4	6.03	9.90	0.51	5.3	3.05
15	24	32	140/90	80	189	264	25	111.2	52.8	7.56	10.56	0.47	4	4.45
16	25	28	140/90	64	182	333	40	75.4	66.6	4.55	8.33	0.60	3.7	1.89
17	22	32	140/90	70	187	254	40	96.2	50.8	4.68	6.35	0.79	4.7	2.41
18	22	28	140/94	75	230	250	29	151	50	7.93	8.62	0.58	3.6	5.21
19	22	32	140/90	68	206	286	39	109.8	57.2	5.28	7.33	0.68	4	2.82
20	23	24	144/90	68	197	294	57	81.2	58.8	3.46	5.16	0.97	4.9	1.42
21	20	32	140/100	70	272	316	38	170.8	63.2	7.16	8.32	0.60	4.6	4.49
22	23	32	140/90	90	268	455	20	157	91	13.40	22.75	0.22	4.2	7.85

23	24	28	140/100	98	275	406	38	155.8	81.2	7.24	10.68	0.47	9	4.10
24	25	34	140/96	100	207	270	45	108	54	4.60	6.00	0.83	5.4	2.40
25	25	34	150/100	100	196	261	38	105.8	52.2	5.16	6.87	0.73	6.8	2.78
26	20	32	140/100	100	223	290	50	115	58	4.46	5.80	0.86	4	2.30
27	32	24-26	160/100	100	180	299	40	80.2	59.8	4.50	7.48	0.67	9.3	2.01
28	25	26	150/100	98	194	291	40	95.8	58.2	4.85	7.28	0.69	4.7	2.40
29	22	26-28	140/94	98	192	294	30	103.2	58.8	6.40	9.80	0.51	3.2	3.44
30	24	28-30	140/90	86	165	280	28	81	56	5.89	10.00	0.50	3.9	2.89
31	25	32	144/90	90	206	370	35	97	74	5.89	10.57	0.47	9.7	2.77
32	23	28	140/94	98	188	281	31	100.8	56.2	6.06	9.06	0.55	5.46	3.25
33	23	20-22	140/94	66	187	301	46	80.8	60.2	4.07	6.54	0.76	5.04	1.76
34	26	24	140/90	68	192	284	27	108.2	56.8	7.11	10.52	0.48	2.52	4.01
35	25	28-30	140/96	104	150	300	47	43	60	3.19	6.38	0.78	4.62	0.91
36	20	32	140/90	76	193	292	44	90.6	58.4	4.39	6.64	0.75	1.68	2.06
37	22	24-26	140/90	82	179	315	60	56	63	2.98	5.25	0.95	2.1	0.93
38	32	28-30	140/94	68	228	280	37	135	56	6.16	7.57	0.66	2.1	3.65
39	25	28	140/94	90	153	340	33	52	68	4.64	10.30	0.49	3.78	1.58
40	20	28-30	140/90	90	176	367	40	62.6	73.4	4.40	9.18	0.54	2.94	1.57
41	22	32	140/98	74	215	314	50	102.2	62.8	4.30	6.28	0.80	3.78	2.04
42	34	24	140/90	62	180	329	38	76.2	65.8	4.74	8.66	0.58	9.4	2.01
43	25	28	140/90	98	251	394	38	134.2	78.8	6.61	10.37	0.48	5.46	3.53
44	23	24	140/94	90	252	286	28	166.8	57.2	9.00	10.21	0.49	5.3	5.96
45	`23	28	140/90	94	202	250	37	115	50	5.46	6.76	0.74	4.8	3.11
46	21	32	140/90	90	241	377	35	130.6	75.4	6.89	10.77	0.46	7.2	3.73
47	23	28	140/100	94	191	430	50	55	86	3.82	8.60	0.58	8.6	1.10
48	24	32	140/96	98	276	361	37	166.8	72.2	7.46	9.76	0.51	6.9	4.51
49	23	32	140/90	68	185	367	36	75.6	73.4	5.14	10.19	0.49	7	2.10

50	27	28	142/100	72	200	396	30	90.8	79.2	6.67	13.20	0.38	7.8	3.03
51	25	32	144/90	90	206	370	35	97	74	5.89	10.57	0.47	9.7	2.77
52	23	20-22	140/94	66	187	301	46	80.8	60.2	4.07	6.54	0.76	5.04	1.76
53	25	28-30	140/96	104	150	300	47	43	60	3.19	6.38	0.78	4.62	0.91
54	22	24-26	140/90	82	179	315	60	56	63	2.98	5.25	0.95	2.1	0.93
55	25	28	140/94	90	153	340	33	52	68	4.64	10.30	0.49	3.78	1.58
56	23	32	140/90	68	185	367	36	75.6	73.4	5.14	10.19	0.49	7	2.10
57	23	28	140/100	94	191	430	50	55	86	3.82	8.60	0.58	8.6	1.10
58	`23	28	140/90	94	202	250	37	115	50	5.46	6.76	0.74	4.8	3.11
59	25	28	140/90	98	251	394	38	134.2	78.8	6.61	10.37	0.48	5.46	3.53
60	22	32	140/98	74	215	314	50	102.2	62.8	4.30	6.28	0.80	3.78	2.04
61	20	32	140/100	70	272	316	38	170.8	63.2	7.16	8.32	0.60	4.6	4.49
62	24	28	140/100	98	275	406	38	155.8	81.2	7.24	10.68	0.47	9	4.10
63	25	34	150/100	100	196	261	38	105.8	52.2	5.16	6.87	0.73	6.8	2.78
64	32	24-26	160/100	100	180	299	40	80.2	59.8	4.50	7.48	0.67	9.3	2.01
65	22	26-28	140/94	98	192	294	30	103.2	58.8	6.40	9.80	0.51	3.2	3.44
66	33	32	144/92	83.5	181	280	40	85	56	4.53	7.00	0.71	4.1	2.13
67	20	32	140/90	57	258	373	28	155.4	74.6	9.21	13.32	0.38	4.4	5.55
68	27	32	140/90	79	249	328	36	147.4	65.6	6.92	9.11	0.55	4.7	4.09
69	28	32	140/94	104	210	520	35	71	104	6.00	14.86	0.34	3.8	2.03
70	22	20	140/90	98	190	400	37	73	80	5.14	10.81	0.46	3	1.97
71	22	32	140/90	68	206	286	39	109.8	57.2	5.28	7.33	0.68	4	2.82
72	22	32	140/90	70	187	254	40	96.2	50.8	4.68	6.35	0.79	4.7	2.41
73	24	32	140/90	80	189	264	25	111.2	52.8	7.56	10.56	0.47	4	4.45
74	24	28	140/100	100	244	480	35	113	96	6.97	13.71	0.36	4.7	3.23
75	26	32	140/90	102	188	540	34	46	108	5.53	15.88	0.31	8.5	1.35
76	27	28	142/100	72	200	396	30	90.8	79.2	6.67	13.20	0.38	7.8	3.03

77	24	32	140/96	98	276	361	37	166.8	72.2	7.46	9.76	0.51	6.9	4.51
78	21	32	140/90	90	241	377	35	130.6	75.4	6.89	10.77	0.46	7.2	3.73
79	23	24	140/94	90	252	286	28	166.8	57.2	9.00	10.21	0.49	5.3	5.96
80	34	24	140/90	62	180	329	38	76.2	65.8	4.74	8.66	0.58	9.4	2.01
81	20	28-30	140/90	90	176	367	40	62.6	73.4	4.40	9.18	0.54	2.94	1.57
82	25	32	150/120	91	232	580	40	76	116	5.80	14.50	0.34	7.6	1.90
83	28	22	140/90	83	261	290	42	161	58	6.21	6.90	0.72	3.7	3.83
84	23	32	140/90	82	252	290	40	154	58	6.30	7.25	0.69	4.1	3.85
85	25	28	140/100	110	214	268	38	122.4	53.6	5.63	7.05	0.71	3.1	3.22
86	28	28	140/90	73.6	216	299	40	116.2	59.8	5.40	7.48	0.67	2.8	2.91
87	24	28-30	140/90	86	165	280	28	81	56	5.89	10.00	0.50	3.9	2.89
88	23	28	140/94	98	188	281	31	100.8	56.2	6.06	9.06	0.55	5.46	3.25
89	26	24	140/90	68	192	284	27	108.2	56.8	7.11	10.52	0.48	2.52	4.01
90	20	32	140/90	76	193	292	44	90.6	58.4	4.39	6.64	0.75	1.68	2.06
91	32	28-30	140/94	68	228	280	37	135	56	6.16	7.57	0.66	2.1	3.65
92	27	32	140/90	86	193	440	30	75	88	6.43	14.67	0.34	4.4	2.50
93	27	24	140/90	81	181	297	30	91.6	59.4	6.03	9.90	0.51	5.3	3.05
94	25	28	140/90	64	182	333	40	75.4	66.6	4.55	8.33	0.60	3.7	1.89
95	22	28	140/94	75	230	250	29	151	50	7.93	8.62	0.58	3.6	5.21
96	23	24	144/90	68	197	294	57	81.2	58.8	3.46	5.16	0.97	4.9	1.42
97	25	26	150/100	98	194	291	40	95.8	58.2	4.85	7.28	0.69	4.7	2.40
98	20	32	140/100	100	223	290	50	115	58	4.46	5.80	0.86	4	2.30
99	25	34	140/96	100	207	270	45	108	54	4.60	6.00	0.83	5.4	2.40
100	23	32	140/90	90	268	455	20	157	91	13.40	22.75	0.22	4.2	7.85

CONTROL														
Sl.No	**AGE**	**LMP (WEEKS)**	**BP**	**FBS**	**TC**	**TGL**	**HDL**	**LDL**	**VLDL**	**TC/HDL**	**TGL/HDL**	**HDL/VLDL**	**UA**	**LDL/HDL**
1	25	28	110/90	**112**	**200**	**188**	**40**	**122.4**	**37.6**	5.00	4.70	1.06	4.3	3.06
2	22	28	110/70	79	177	230	34	97	46	5.21	6.76	0.74	3.4	2.85
3	21	32	120/80	107	157	200	40	77	40	3.93	5.00	1.00	4.7	1.93
4	18	28	130/90	70	173	180	31	106	36	5.58	5.81	0.86	3	3.42
5	22	24	120/80	65	191	200	40	111	40	4.78	5.00	1.00	3.2	2.78
6	23	28-30	120/70	101	189	210	42	105	42	4.50	5.00	1.00	5.3	2.50
7	20	28-30	110/80	100	185	149	46	109.2	29.8	4.02	3.24	1.54	3.9	2.37
8	24	28	120/70	98	172	212	40	89.6	42.4	4.30	5.30	0.94	3.4	2.24
9	24	26-28	120/70	80	200	137	40	132.6	27.4	5.00	3.43	1.46	3.4	3.32
10	25	28	110/70	102	175	177	56	83.6	35.4	3.13	3.16	1.58	4.5	1.49
11	22	28	110/70	100	197	176	47	114.8	35.2	4.19	3.74	1.34	2.1	2.44
12	20	24	110/70	96	149	200	60	49	40	2.48	3.33	1.50	3.2	0.82
13	26	28-30	120/70	104	115	114	60	32.2	22.8	1.92	1.90	2.63	4.2	0.54
14	32	26-28	130/80	100	189	251	43	95.8	50.2	4.40	5.84	0.86	3	2.23
15	24	24-26	110/70	98	160	180	47	77	36	3.40	3.83	1.31	2.14	1.64
16	23	20-22	120/70	104	174	233	43	84.4	46.6	4.05	5.42	0.92	3	1.96
17	27	28-30	110/70	84	171	156	52	87.8	31.2	3.29	3.00	1.67	3	1.69
18	21	32	120/70	70	110	210	45	23	42	2.44	4.67	1.07	2.57	0.51
19	25	28	110/70	68	160	157	42	86.6	31.4	3.81	3.74	1.34	2.57	2.06
20	23	24-26	120/70	70	195	215	60	92	43	3.25	3.58	1.40	4.2	1.53
21	25	22-24	110/70	90	163	187	55	70.6	37.4	2.96	3.40	1.47	2.1	1.28
22	25	24	130/90	100	209	267	47	108.6	53.4	4.45	5.68	0.88	3.36	2.31
23	24	24-26	130/90	96	240	222	58	137.6	44.4	4.14	3.83	1.31	5.04	2.37
24	23	28	120/80	78	163	216	54	65.8	43.2	3.02	4.00	1.25	2.52	1.22

25	35	26-28	120/70	98	135	188	40	57.4	37.6	3.38	4.70	1.06	2.94	1.44
26	28	32	130/90	100	123	232	50	26.6	46.4	2.46	4.64	1.08	3.78	0.53
27	20	24-26	110/70	76	126	171	47	44.8	34.2	2.68	3.64	1.37	3.78	0.95
28	28	24	120/80	96	160	125	46	89	25	3.48	2.72	1.84	2.1	1.93
29	21	28-30	130/90	70	187	260	50	85	52	3.74	5.20	0.96	4.2	1.70
30	25	28-30	130/80	98	208	236	32	128.8	47.2	6.50	7.38	0.68	3.8	4.03
31	28	24-26	110/70	74	200	186	55	107.8	37.2	3.64	3.38	1.48	3.6	1.96
32	23	28-30	120/70	96	206	158	40	134.4	31.6	5.15	3.95	1.27	2.9	3.36
33	21	28	110/80	80	186	160	40	114	32	4.65	4.00	1.25	3.78	2.85
34	32	28	110/70	98	216	204	58	117.2	40.8	3.72	3.52	1.42	5.1	2.02
35	23	28	110/70	68	160	165	52	75	33	3.08	3.17	1.58	4.5	1.44
36	20	22-24	110/*70	90	191	156	49	110.8	31.2	3.90	3.18	1.57	3.6	2.26
37	28	30-32	120/70	86	180	162	64	83.6	32.4	2.81	2.53	1.98	4.8	1.31
38	20	28	110/80	104	200	168	57	109.4	33.6	3.51	2.95	1.70	2.9	1.92
39	23	28	110/70	70	162	165	55	74	33	2.95	3.00	1.67	3.8	1.35
40	23	24	120/80	76	158	191	55	64.8	38.2	2.87	3.47	1.44	3.56	1.18
41	24	24	130/90	62	197	273	52	90.4	54.6	3.79	5.25	0.95	2.75	1.74
42	29	32	120/70	72	104	193	48	17.4	38.6	2.17	4.02	1.24	2.7	0.36
43	24	32	130/90	90	140	246	36	54.8	49.2	3.89	6.83	0.73	4.2	1.52
44	21	30	110/80	92	186	212	40	103.6	42.4	4.65	5.30	0.94	3.6	2.59
45	19	32	130/90	78	180	200	50	90	40	3.60	4.00	1.25	3.8	1.80
46	20	32	110/90	80	146	190	55	53	38	2.65	3.45	1.45	3.7	0.96
47	26	28	120/80	88	156	190	45	73	38	3.47	4.22	1.18	3.8	1.62
48	27	32	120/80	96	191	258	45	94.4	51.6	4.24	5.73	0.87	5	2.10
49	20	32	110/80	110	246	254	39	156.2	50.8	6.31	6.51	0.77	3.2	4.01
50	23	32	110/70	90	200	264	53	94.2	52.8	3.77	4.98	1.00	4.8	1.78

51	28	24-26	110/70	74	200	186	55	107.8	37.2	3.64	3.38	1.48	3.6	1.96
52	21	28	110/80	80	186	160	40	114	32	4.65	4.00	1.25	3.78	2.85
53	23	28	110/70	68	160	165	52	75	33	3.08	3.17	1.58	4.5	1.44
54	28	30-32	120/70	86	180	162	64	83.6	32.4	2.81	2.53	1.98	4.8	1.31
55	23	28	110/70	70	162	165	55	74	33	2.95	3.00	1.67	3.8	1.35
56	20	32	110/80	110	246	254	39	156.2	50.8	6.31	6.51	0.77	3.2	4.01
57	26	28	120/80	88	156	190	45	73	38	3.47	4.22	1.18	3.8	1.62
58	19	32	130/90	78	180	200	50	90	40	3.60	4.00	1.25	3.8	1.80
59	24	32	130/90	90	140	246	36	54.8	49.2	3.89	6.83	0.73	4.2	1.52
60	24	24	130/90	62	197	273	52	90.4	54.6	3.79	5.25	0.95	2.75	1.74
61	25	22-24	110/70	90	163	187	55	70.6	37.4	2.96	3.40	1.47	2.1	1.28
62	24	24-26	130/90	96	240	222	58	137.6	44.4	4.14	3.83	1.31	5.04	2.37
63	35	26-28	120/70	98	135	188	40	57.4	37.6	3.38	4.70	1.06	2.94	1.44
64	20	24-26	110/70	76	126	171	47	44.8	34.2	2.68	3.64	1.37	3.78	0.95
65	21	28-30	130/90	70	187	260	50	85	52	3.74	5.20	0.96	4.2	1.70
66	25	28	110/90	112	200	188	40	122.4	37.6	5.00	4.70	1.06	4.3	3.06
67	21	32	120/80	107	157	200	40	77	40	3.93	5.00	1.00	4.7	1.93
68	22	24	120/80	65	191	200	40	111	40	4.78	5.00	1.00	3.2	2.78
69	20	28-30	110/80	100	185	149	46	109.2	29.8	4.02	3.24	1.54	3.9	2.37
70	24	26-28	120/70	80	200	137	40	132.6	27.4	5.00	3.43	1.46	3.4	3.32
71	25	28	110/70	68	160	157	42	86.6	31.4	3.81	3.74	1.34	2.57	2.06
72	27	28-30	110/70	84	171	156	52	87.8	31.2	3.29	3.00	1.67	3	1.69
73	24	24-26	110/70	98	160	180	47	77	36	3.40	3.83	1.31	2.14	1.64
74	26	28-30	120/70	104	115	114	60	32.2	22.8	1.92	1.90	2.63	4.2	0.54
75	22	28	110/70	100	197	176	47	114.8	35.2	4.19	3.74	1.34	2.1	2.44
76	23	32	110/70	90	200	264	53	94.2	52.8	3.77	4.98	1.00	4.8	1.78

77	27	32	120/80	96	191	258	45	94.4	51.6	4.24	5.73	0.87	5	2.10
78	20	32	110/90	80	146	190	55	53	38	2.65	3.45	1.45	3.7	0.96
79	21	30	110/80	92	186	212	40	103.6	42.4	4.65	5.30	0.94	3.6	2.59
80	29	32	120/70	72	104	193	48	17.4	38.6	2.17	4.02	1.24	2.7	0.36
81	23	24	120/80	76	158	191	55	64.8	38.2	2.87	3.47	1.44	3.56	1.18
82	25	28	110/70	102	175	177	56	83.6	35.4	3.13	3.16	1.58	4.5	1.49
83	24	28	120/70	98	172	212	40	89.6	42.4	4.30	5.30	0.94	3.4	2.24
84	23	28-30	120/70	101	189	210	42	105	42	4.50	5.00	1.00	5.3	2.50
85	18	28	130/90	70	173	180	31	106	36	5.58	5.81	0.86	3	3.42
86	22	28	110/70	79	177	230	34	97	46	5.21	6.76	0.74	3.4	2.85
87	25	28-30	130/80	98	208	236	32	128.8	47.2	6.50	7.38	0.68	3.8	4.03
88	23	28-30	120/70	96	206	158	40	134.4	31.6	5.15	3.95	1.27	2.9	3.36
89	32	28	110/70	98	216	204	58	117.2	40.8	3.72	3.52	1.42	5.1	2.02
90	20	22-24	110/*70	90	191	156	49	110.8	31.2	3.90	3.18	1.57	3.6	2.26
91	20	28	110/80	104	200	168	57	109.4	33.6	3.51	2.95	1.70	2.9	1.92
92	20	24	110/70	96	149	200	60	49	40	2.48	3.33	1.50	3.2	0.82
93	32	26-28	130/80	100	189	251	43	95.8	50.2	4.40	5.84	0.86	3	2.23
94	23	20-22	120/70	104	174	233	43	84.4	46.6	4.05	5.42	0.92	3	1.96
95	21	32	120/70	70	110	210	45	23	42	2.44	4.67	1.07	2.57	0.51
96	23	24-26	120/70	70	195	215	60	92	43	3.25	3.58	1.40	4.2	1.53
97	28	24	120/80	96	160	125	46	89	25	3.48	2.72	1.84	2.1	1.93
98	28	32	130/90	100	123	232	50	26.6	46.4	2.46	4.64	1.08	3.78	0.53
99	23	28	120/80	78	163	216	54	65.8	43.2	3.02	4.00	1.25	2.52	1.22
100	25	24	130/90	100	209	267	47	108.6	53.4	4.45	5.68	0.88	3.36	2.31

TABLE - 1

COMPARISON OF TOTAL CHOLESTEROL BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 1	TC in PIH	209.54	34.380	43.849	4.385	25.679	43.081	7.841	99	(P<0.0001)
	TC in Control	175.16								

Total Cholesterol is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 2

COMPARISON OF TRIGLYCERIDES BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 2	TGL in PIH TGL in Control	337.24 197.48	139.760	88.432	8.843	122.213	157.307	15.804	99	(P<0.0001)

Triglycerides is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 3

COMPARISON OF HDL BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 3	HDL in PIH HDL in Control	37.62 47.50	-9.880	9.679	.968	-11.801	-7.959	-10.208	99	(P<0.0001)

HDL is significantly decreased in PIH group compared to control ($p < 0.0001$)

TABLE - 4

COMPARISON OF LDL BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 4	LDL in PIH LDL in Control	104.47 88.164	16.308	46.285	4.629	7.124	25.492	3.523	99	(P<0.0001)

LDL is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 5

COMPARISON OF VLDL BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 5	VLDL in PIH VLDL in Control	67.45 39.496	27.952	17.687	1.769	24.443	31.461	15.804	99	(P<0.0001)

VLDL is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 6

COMPARISON OF TC/HDL RATIO BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 6	TC/HDL in PIH TC/HDL in Control	5.843 3.81	2.0379	1.881	.188	1.665	2.411	10.833	99	(P<0.0001)

TC/HDL Ratio is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 7

COMPARISON OF TGL/HDL RATIO BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 7	TGL/HDL in PIH TGL/HDL in Control	9.41 4.302	5.110	3.437	.3437	4.428	5.792	14.866	99	(P<0.0001)

TGL/HDL Ratio is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 8

COMPARISON OF HDL/VLDL BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 8	HDL/ VLDL in PIH	.5846	-.673	.397	.040	-.751	-.594	-16.941	99	(P<0.0001)
	HDL/ VLDL in Control	1.2571								

HDL/VLDL is significantly decreased in PIH group compared to control ($p < 0.0001$)

TABLE - 9

COMPARISON OF URIC ACID BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 9	UA in PIH UA in Control	5.008 3.576	1.432	2.164	.216	1.002	1.861	6.616	99	(P<0.0001)

Uric Acid is significantly elevated in PIH group compared to control ($p < 0.0001$)

TABLE - 10

COMPARISON OF LDL/HDL RATIO BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 10	LDL/HDL in PIH LDL/HDL in Control	2.961 1.945	1.016	1.543	.154	.710	1.322	6.584	99	(P<0.001)

LDL/HDL in PIH is significantly elevated in PIH group compared to control (p< 0.0001)

TABLE - 11

COMPARISON OF FBS BETWEEN PIH AND CONTROL GROUPS

			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Upper	Lower			
Pair 11	FBS in PIH FBS in Control	85.342 88.20	-2.8580	19.31	1.930	-6.690	.9736	-1.480	99	(P>0.001)

FBS in PIH is not significantly altered, when compared to FBS in control (P> 0.0001)

CORRELATIONS

		TC	TGL	HDL	LDL	VLDL	TC/HDL	HDL/VLDL	UA
TC	Pearson Correlation	1	.206(*)	-.172	.884(**)	.206(*)	.664(**)	-.241(*)	.072
	Sig. (2-tailed)		.040	.087	.000	.040	.000	.016	.477
	N	100	100	100	100	100	100	100	100
TGL	Pearson Correlation	.206(*)	1	-.137	-.223(*)	1.000(**)	.223(*)	-.697(**)	.370(**)
	Sig. (2-tailed)	.040		.174	.026	.000	.026	.000	.000
	N	100	100	100	100	100	100	100	100
HDL	Pearson Correlation	-.172	-.137	1	-.323(**)	-.137	-.774(**)	.786(**)	-.038
	Sig. (2-tailed)	.087	.174		.001	.174	.000	.000	.708
	N	100	100	100	100	100	100	100	100
LDL	Pearson Correlation	.884(**)	-.223(*)	-.323(**)	1	-.223(*)	.695(**)	-.090	-.090
	Sig. (2-tailed)	.000	.026	.001		.026	.000	.371	.376
	N	100	100	100	100	100	100	100	100
VLDL	Pearson Correlation	.206(*)	1.000(**)	-.137	-.223(*)	1	.223(*)	-.697(**)	.370(**)
	Sig. (2-tailed)	.040	.000	.174	.026		.026	.000	.000
	N	100	100	100	100	100	100	100	100
TC/ HDL	Pearson Correlation	.664(**)	.223(*)	-.774(**)	.695(**)	.223(*)	1	-.664(**)	.006
	Sig. (2-tailed)	.000	.026	.000	.000	.026		.000	.949
	N	100	100	100	100	100	100	100	100
HDL/VLDL	Pearson Correlation	-.241(*)	-.697(**)	.786(**)	-.090	-.697(**)	-.664(**)	1	-.277(**)
	Sig. (2-tailed)	.016	.000	.000	.371	.000	.000		.005
	N	100	100	100	100	100	100	100	100
UA	Pearson Correlation	.072	.370(**)	-.038	-.090	.370(**)	.006	-.277(**)	1
	Sig. (2-tailed)	.477	.000	.708	.376	.000	.949	.005	
	N	100	100	100	100	100	100	100	100

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Correlations between the various lipid fractions in PIH Patients :

1. There is significant positive correlation between Total Cholesterol and TGL, VLDL, LDL, TC/HDL ratio in pregnancy induced Hypertensive patients.

   There is significant negative correlation between Total Cholesterol and HDL/VLDL ratio(i.e.) if Total cholesterol is increased in PIH the ratio of HDL /VLDL is decreased.

2. The Triglyceride fraction is positively correlated with VLDL, Uric Acid & TC / HDL ratio and negatively correlated with HDL/VLDL ratio and both are highly significant.

3. There is significant positive correlation between VLDL and TGL, UA, TC / HDL ratio which is highly significant.

4. HDL fraction is positively correlated with HDL/VLDL ratio and negatively correlated with LDL and TC/HDL ratio and both are highly significant.

FACTOR ANALYSIS

Rotated Component Matrix(a)

	Component		
	1	**2**	**3**
TC	.085	.215	.963
TGL	.295	.928	-.050
HDL	-.949	.098	-.140
LDL	.159	-.235	.956
VLDL	.295	.928	-.050
TC/HDL	.751	.045	.627
TGL/HDL	.799	.556	.083
HDL/VLDL	-.841	-.498	-.065
UA	-.090	.615	.052

Total Variance Explained

Components		**Rotation Sums of Squared Loadings**	
	Total	**% of Variance**	**Cumulative %**
1	3.024	33.604	33.604
2	2.771	30.791	64.396
3	2.272	25.248	89.644

1. TGL/HDL
2. TGL or VLDL
3. TC

The 3 factors accounted for 89.644 percent of the variance in the original nine variables.

TGL/HDL, TGL or VLDL & TC contribute more to the Pregnancy induced Hypertension.

DISCUSSION

There is dramatic alteration in lipid profile which has been elevated since 20 weeks of gestation.

From Table-1 we infer that total cholesterol is significantly elevated in women with Pregnancy Induced Hypertension when compared to normotensive pregnant women.

Table-II shows the highly significant elevation of serum triglycerides in women with Pregnancy Induced Hypertensive than in normotensive pregnant women.

Table-III shows the significant decrease in the serum level of high density lipoprotein in women with Pregnancy Induced Hypertension when compared to normotensive pregnant women.

Table-IV & V shows the highly significant elevation of low density lipoprotein and very low density lipoprotein respectively in women with Pregnancy Induced Hypertension.

Pregnancy induced hypertension is a state of hypoestrogenemia[8,75]. Decreased uteroplacental blood flow which is the main pathophysiological event in pregnancy induced hypertension leads to impairment in the formation of Dehydroepiandrosterone sulphate by fetal adrenal glands

1) DHEA.is the important source of estrogen in pregnancy,(i.e),90% of estrogen in maternal circulation is from fetal dehydroepiandrosterone sulphate which is converted to estriol in placenta.[93] Dehydroepiandrosteronesulphate on desulphuration and aromatisation by the enzymes sulphatase and aromatase in the placenta forms estriol.[97]

$$\text{DHEAs} \xrightarrow[\text{Aromatase}]{\text{Sulphatase}} \text{estriol}$$

Because of the impairment in the formation of dehydroepiandrosterone estrogen levels decrease.

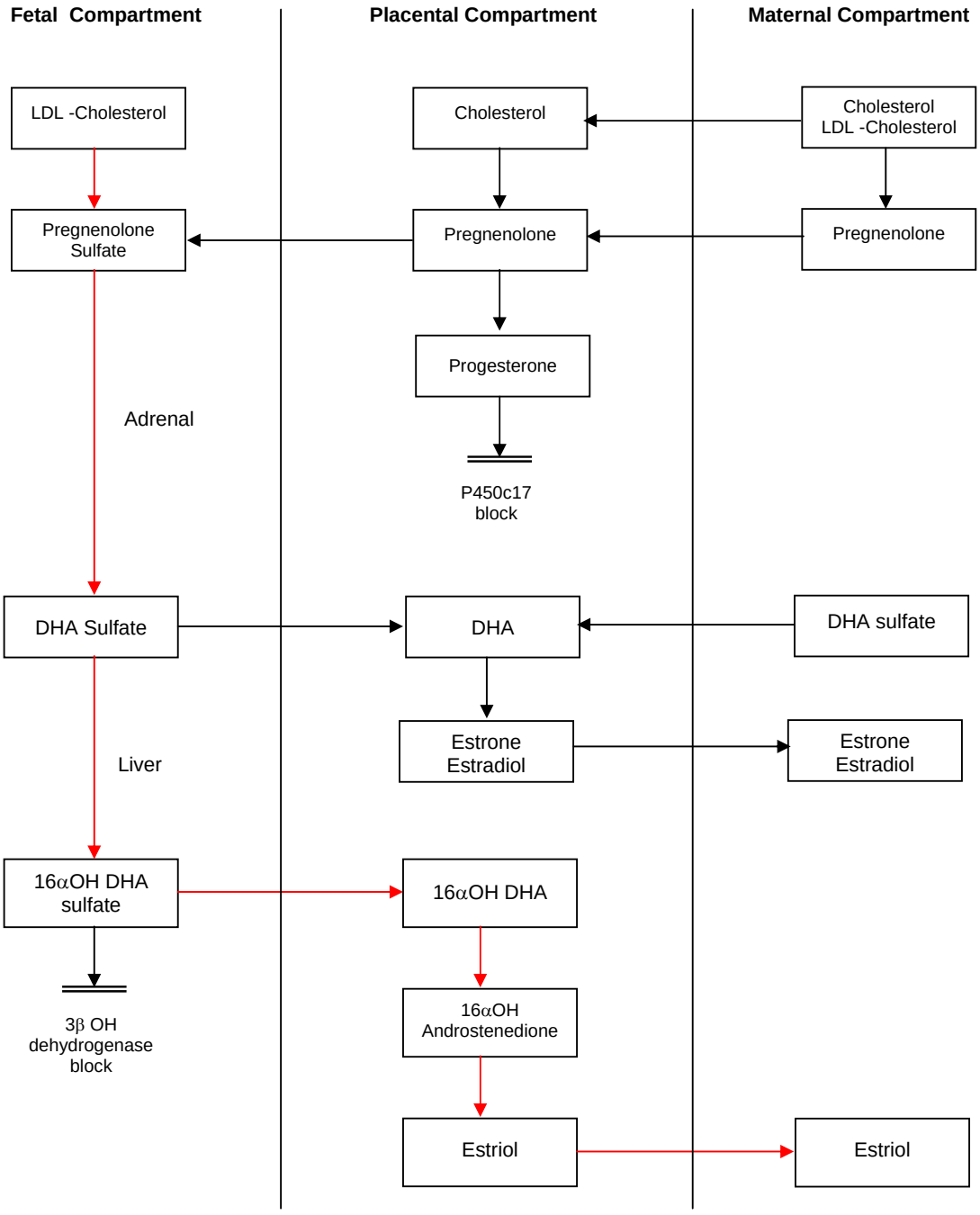
↓Uteroplacental perfusion → ↓Lipid uptake by fetus → ↓Synthesis of DHEAs ↓ Estriol ↓ ↓

Moreover decrease in uteroplacental blood flow leads to relative stasis of maternal blood in the intervillous space resulting in redistribution of steroids formed in syncitium back to the fetus.

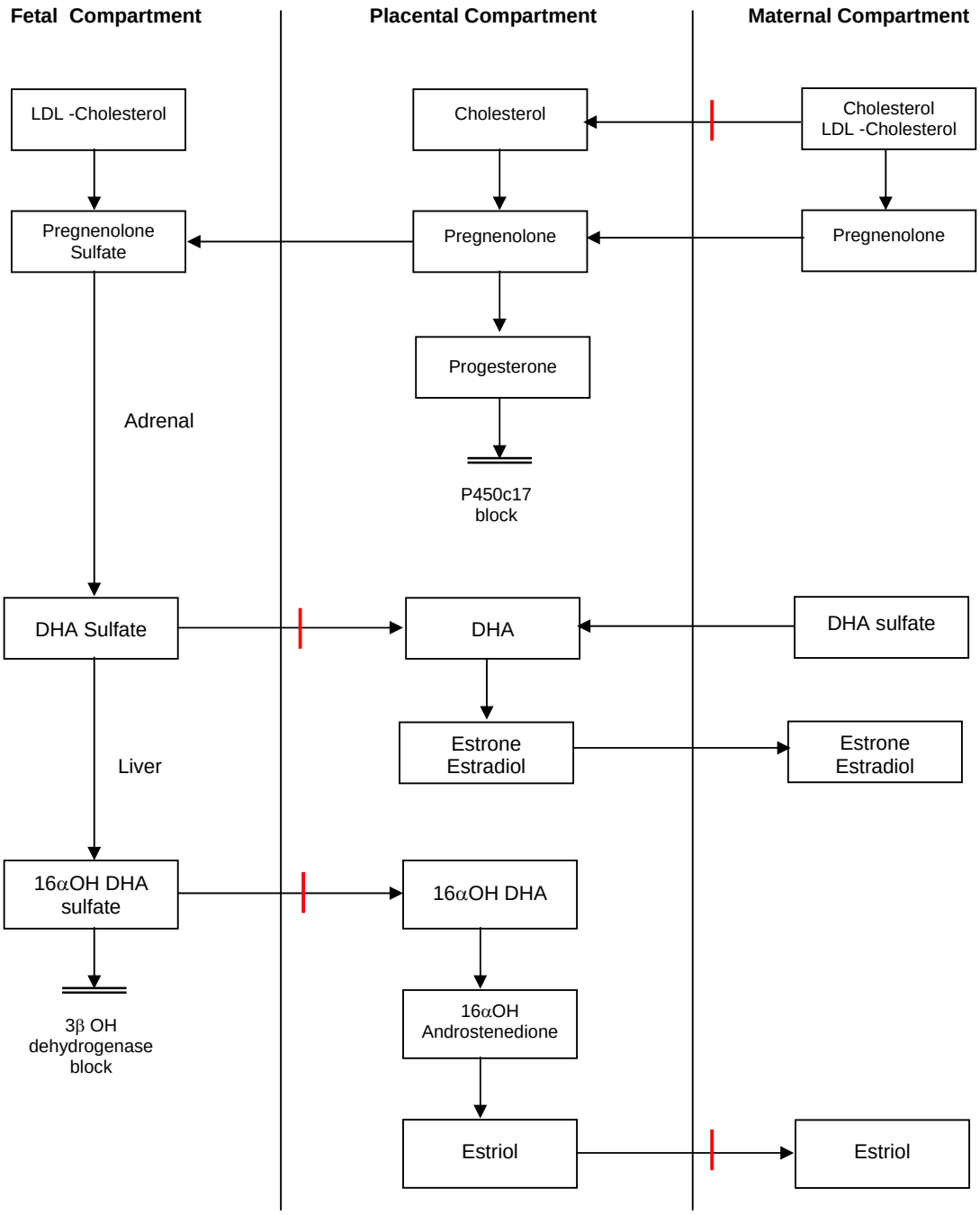
↓Uteroplacental blood flow → Relative stasis of maternal blood in the intervillous space ↓ Redistribution of steroids formed in syncitium back to the fetus instead of entering into maternal circulation.

Hence a state of hypoestrogenemia develops.[97]

IN NORMAL PREGNANCY



IN PIH DUE TO PLACENTAL IN SUFFICIENCY



Since estrogen level is decreased after the placenta has taken over the function, there will be no decrease in the activities of lipoprotein lipase and hepatic lipase as in normal pregnancy,which is a state of hyperestrogenemia.This leads to increased levels of free fatty acids.

Free fatty acids are essential for the fetus for its energy needs. The very long chain fatty acids are synthesized by the fetal liver and brain from maternal linoleic and linolinic acids.

Further placental transport of fatty acids from maternal compartment is by simple diffusion but its capacity is limited. So for, lipid transfer across placenta fetus depends mainly upon VLDL/apo E receptors in the placenta. The expression of these receptors are increased by placental hormones but here in PIH,decreased estrogen leads to decreased expression of VLDL/apo E receptors resulting in reduced transport of VLDL to fetal compartment so there is maternal hypertriglycerimedia.

Hypoestrogenemia ⟶ Decreased expression of VLDL /apoE receptors.

↓

Decreased transport of lipids to fetal compartment

↓

Increased maternal hypertriglyceridemia

Further LDL taken up by the fetus for the synthesis of DHEA is decreased due to reduced fetoplacental perfusion leading to increased LDL.

Genetic Basis

Some studies show that mutation in LPL gene in pre-eclampsia may lead to lowered activity of LPL resulting in decreased hydrolysis of Triglycerides and predispose to dyslipidemia and cardiovascular disease[91].

There are 3 common mutations in LPL gene,

(1) Asp9Asn substitution in exon 2,

(2) T-to-G substitution at position -93 of the proximal promotor region (-93T/G), and

(3) Asn291Ser substitution in exon 6.

The LPL Asp9Asn mutation is in nonrandom association with a T-to-G substitution at position -93 of the proximal promotor region, and the combined -93G/Asn9 genotype predisposes to decreased HDL cholesterol and an increased risk of coronary arterydisease.[94].

Recently, a more common serine for asparagine substitution at residue 291 (Asn291Ser) in exon 6 of the LPL gene has been described that is observed with high frequency, ranging from 2% to 5% in different populations.[9] This common Asn291Ser LPL mutation significantly influences the risk for cardiovascular disease in patients with familialhypercholesterolemia[95]

There is also evidence that there is active role of placental LPL and Apo E in the metabolism of maternal lipoproteins and so fetal genes may modulate the risk for problems related to maternal dyslipidemia.[96]

Genetic polymorphisms present in newborns are associatedwith variations of lipid concentrations in their mothers.

1) The presence of the S447X allele of LPL in newborns was associated with lower triglycerides, lower LDL-C, lower apoB, higher HDL-C, and higher apoA-I in their mothers,
2) The presence of the N291S allele of LPL in newborns was associated with higher triglycerides in their mothers.
3) The presence of the E2 allele of APOE in newborns was associated with higher LDL-C and apoB in their mothers.[94]

In late gestation, high amounts of TGs are found not only in VLDL but also in IDL, LDL, and HDL[78,79.] This is because of the reduced removal of lipoprotein TGs due to the decreased activities of lipoprotein lipase (LPL) and hepatic lipase (HL), the effect being more striking for HL than for LPL [80,81].

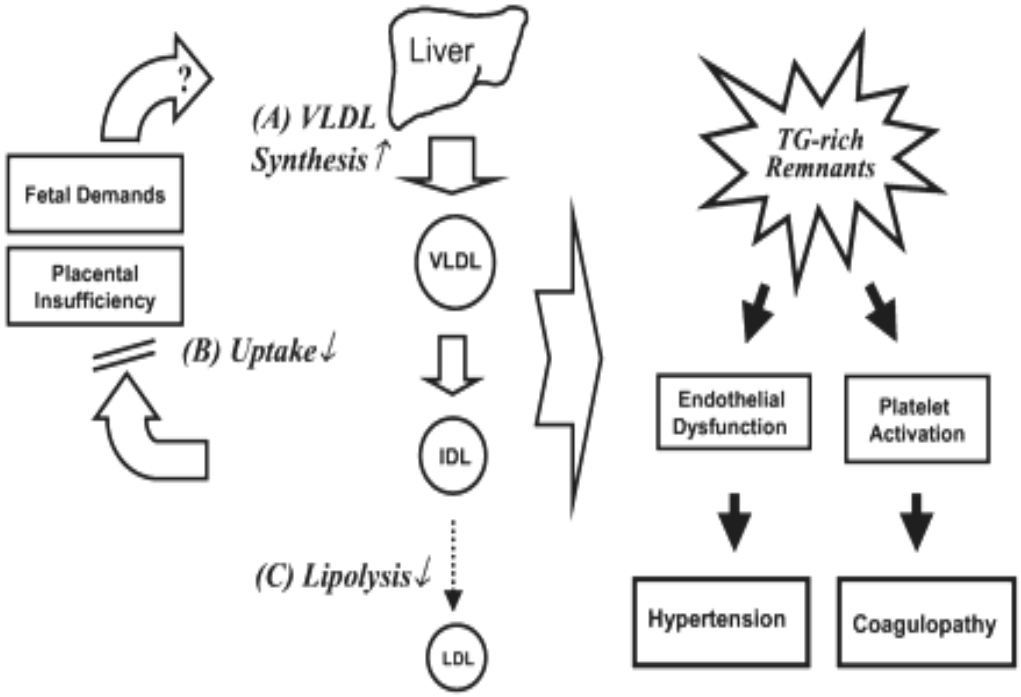
Insufficient lipolysis of TG-rich lipoproteins leads to the accumulation of remnant lipoproteins, which are otherwise quickly removed from the circulation, with an increased TG content and prolonged residence time.

The changes in lipoprotein metabolism observed in PIH closely resemble those seen in HL deficiency.

In the late phase of normal pregnancy, estrogen enhances the production of VLDLs and decreases maternal lipolytic activity[80,81]. Together with the increased expression of the VLDL/apoE receptors in the placenta[82], this may result in a coordinated rerouting of TG-rich lipoproteins from the mother toward the fetoplacental unit to meet the nutritional demands of the growing fetus[78].

But in pre-eclampsia, the reduced maternal lipolysis and the hampered uptake of TG-rich lipoproteins by the fetoplacental unit lead to the accumulation of TG-rich remnant lipoproteins in the maternal circulation

Remnant accumulation may reflect not only insufficient uptake of these particles by the fetoplacental unit but may also be pivotal to the development of clinical symptoms of pre-eclampsia.



Renal protein excretion and hypertension may both reflect endothelial dysfunction. Arbogast et al. (83) hypothesized that TG-rich lipoproteins from preeclamptic women damage the endothelium. Indeed, remnant lipoproteins are associated with impaired endothelial vasomotor function[77], and earlier studies reported that women with PE have an increased pressure response to angiotensin [76] In line with this, we observed an association of the accumulation of TGs in IDL particles and the depletion of dLDL particles, with diastolic blood pressure and of IDL triglycerides with proteinuria .This suggests that the alteration of the lipoprotein metabolism plays a key role in the development of the primary symptoms of PIH.

CONCLUSION

There is a definite relationship between lipid profile and PIH. Moreover the hormonal imbalance is a prime factor for the aetiopathogenesis of PIH and this endocrinal imbalance is well reflected in alteration of serum lipid profile. Hence simple measurement of lipid profile at 20 weeks of gestation can go a long way in prevention of the complications of PIH like, eclampsia, intrauterine growth retardation, HELLP syndrome future cardio vascular risk of the mother, future development of Hypertension and stroke. A clinical trial of life style and dietary modification would help.[2],in cases of altered lipid metabolism.

Further studies exploring the lipoprotein particles and microparticles as well as detailed analysis of microvascular bed of delivered placenta may address the role of lipid profile in the causation of PIH.

. Thus, our findings may be relevant for the future treatment by lipid modifying regimens of this life-threatening condition, for example, by drug therapy or lipoprotein apheresis

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LIST OF ABBREVIATIONS

AT1	-	Angiotensin II TYPE 1 receptor
ATP	-	Adenosine Tri Phosphate
CAMS	-	Cellular Adhesion Molecules
Enos	-	Endothelial Nitric Oxide Synthase
ET	-	Endothelin
FFA	-	Free Fatty Acid
GFR	-	Glomerular Filtration Rate
HDL	-	High density lipoprotein
HELLP	-	Hemolysis, Elevated liver enzymes, Low platelet Syndrome
ICAM -1	-	Inter cellular Adhesion Molecule -1
IUGR	-	Intra Uterine Growth restriction
LCAT	-	Lecithin Cholesterol Acyl Transferase
LDL	-	Low density lipoprotein
LPL	-	Lipoprotein Lipase
MMP2	-	Matrix Metallo Proteinase 2
NO	-	Nitric Oxide
PG12/TXA2	-	Prostaglandin I2 / Thrombaxane A2
P1GF	-	Placental Growth Factor
PIH	-	Pregnancy induced hypertension
RBF	-	Renal Blood Flow
ROS	-	Reactive Oxygen Species
sflt-1	-	Soluble Fms like tyrosine Kinase -1
TGL	-	Triglycerides
VEGF	-	Vascular Endothelial Growth Factor
VLDL	-	Very low density lipoprotein
VCAM -1	-	Vascular Cellular Adhesion Molecule-1
FBS	-	Fasting Blood Sugar
UA	-	Uric Acid